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Mishra

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(54) **NEUTROPHIL-DEPLETED PLATELET RICH PLASMA FORMULATIONS FOR CARDIAC TREATMENTS**

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(52) **U.S. Cl.**

USPC **424/93.72; 424/93.71; 435/372**

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,414,108 A	11/1983	Ito
4,957,742 A	9/1990	Knighton
5,147,776 A	9/1992	Koerner, Jr.
5,165,938 A	11/1992	Knighton
5,178,883 A	1/1993	Knighton
5,403,272 A	4/1995	Deniega et al.
5,474,891 A	12/1995	Murphy
5,494,590 A	2/1996	Smith et al.
5,510,102 A	4/1996	Cochrum
5,578,460 A	11/1996	Ebersole et al.
5,578,565 A	11/1996	Chao et al.
5,585,007 A	12/1996	Antanavich et al.
5,599,558 A	2/1997	Gordinier et al.
5,614,204 A	3/1997	Cochrum
5,643,786 A	7/1997	Cohen et al.
5,676,849 A	10/1997	Sammons et al.
5,733,545 A *	3/1998	Hood, III 424/93.72
5,773,033 A	6/1998	Cochrum et al.
5,785,869 A	7/1998	Martinson et al.
5,788,662 A	8/1998	Antanavich et al.
5,834,418 A	11/1998	Brazeau et al.
5,916,743 A	6/1999	Lake et al.
5,928,214 A	7/1999	Rubinstein et al.
5,993,804 A	11/1999	Read et al.
6,063,297 A	5/2000	Antanavich et al.
6,098,631 A	8/2000	Holoshitz et al.
6,210,976 B1	4/2001	Sabbadini
6,214,338 B1	4/2001	Antanavich et al.
6,432,119 B1	8/2002	Saadat
6,444,228 B1	9/2002	Baugh et al.
6,596,179 B2	7/2003	Giesler et al.
6,811,777 B2	11/2004	Mishra
6,905,612 B2	6/2005	Dorian et al.
6,942,639 B2	9/2005	Baugh et al.

6,942,880 B1	9/2005	Dolecek et al.
7,169,547 B2	1/2007	Rubinstein et al.
7,211,191 B2	5/2007	Coelho et al.
7,252,758 B2	8/2007	Dolecek et al.
7,314,617 B2	1/2008	Mishra
7,608,258 B2	10/2009	Mishra
7,708,152 B2	5/2010	Dorian et al.
2002/0004038 A1	1/2002	Baugh et al.
2002/0058030 A1	5/2002	Monroy et al.
2002/0147611 A1	10/2002	Greene et al.
2003/0116512 A1	6/2003	Antwiller et al.
2003/0152639 A1	8/2003	Britton et al.
2003/0175248 A1	9/2003	Uhr
2003/0191005 A1	10/2003	Coelho et al.
2003/0205538 A1	11/2003	Dorian et al.
2003/0233064 A1	12/2003	Arm et al.
2003/0233065 A1	12/2003	Steward et al.
2004/0091459 A1*	5/2004	Nimni 424/93.7
2004/0131583 A1	7/2004	Barrault et al.
2004/0182795 A1	9/2004	Dorian et al.
2005/0186193 A1	8/2005	Mishra
2005/0209081 A1	9/2005	Baugh et al.

(Continued)

FOREIGN PATENT DOCUMENTS

EP	0 142-339	5/1985
EP	0 417 818	3/1991

(Continued)

OTHER PUBLICATIONS

Esa, et al. "Immunological Heterogeneity of Human Monocyte Subsets Prepared by Counterflow Centrifugation Elutriation," *Immunology*, vol. 59, pp. 95-99, 1986.

Sharpe, P.T., Chapter 5, *Centrifugal Elutriation*, R.H. Burdon and P.H. van Knippenberg editors, *Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, NL, pp. 91-94, 97-100, 101 and 105, 1998.

Rink, "Cytosolic Calcium in Platelet Activation," *Cellular and Molecular Life Sciences*, vol. 44, No. 2, pp. 97-100, downloaded from <http://www.springerlink.com/content/j41h051h8866m352/?target=print>, Abstract only, Feb. 1988.

Dohan Ehrenfest, et al. "Classification of Platelet Concentrates: from Pure Platelet-rich Plasma (P-PRP) to Leucocyte- and Platelet-rich Fibrin (L-PRF)," *Trends in Biotechnology*, vol. 27, No. 3, pp. 158-167, 2008.

(Continued)

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(57) **ABSTRACT**

Compositions and methods for preparing neutrophil-depleted platelet rich plasma are provided. Generally, these compositions comprise a higher concentration of platelets and depressed concentrations of neutrophils relative to whole blood although white blood cells may be at higher concentrations than whole blood. The concentrations of the platelets and/or the white blood cells may be two to eight times the respective concentrations in whole blood. These compositions may have depressed concentrations of red blood cells and hemoglobin. In some variations, the compositions may be useful to treat damaged connective tissue and/or to slow or stop cardiac apoptosis after a heart attack. The neutrophil-depleted platelet rich plasma composition may be delivered in conjunction with reperfusion therapy.